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**DMMP Clarification Paper**

# AMMONIA AND AMPHIPOD TOXICITY TESTING

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## INTRODUCTION

Over the history of the DMMP, agency staff has not often found evidence of substantial ammonia toxicity interfering with interpretation of sediment toxicity tests. However, it is clear that ammonia remains a potential interference, or non-treatment factor, especially for toxicity tests conducted on deep sediment samples. To address this issue, the agencies recently adopted a recommendation to conduct water-only ammonia reference toxicant ($LC_{50}$ ) tests in parallel with standard amphipod and sediment larval bioassays (DMMP, 2001) in some instances. The ammonia dose-response data that result can then be used to determine whether or not ammonia may have contributed to any observed toxicity.

## PROBLEM IDENTIFICATION

Since the 2001 clarification, two local project proponents expressed concerns about interstitial ammonia potentially interfering with planned amphipod bioassays and proposed to purge samples prior to the initiation of testing, in addition to conducting the water-only $LC_{50}$ test.

The DMMP has little direct experience with ammonia purging procedures, having allowed sample purging for one previous project. The agencies decided to allow limited purging for the two recent projects, should ammonia measured in bulk interstitial water exceed the thresholds established by EPA (1993, 1994). However, the DMMP agencies continue to be reluctant to allow purging of toxicity test samples on a programmatic basis. This is because staff is not aware of careful studies showing that purging reduces only ammonia and does not also reduce the intended exposure to sediment contaminants. Nevertheless, it is likely that there will be occasional sediment samples from DMMP projects where initial bulk interstitial ammonia values indicate the need to purge in order to conduct a reliable toxicity test.

As part of a general effort to document ammonia concentrations and minimize potential toxicity test interference due to high ammonia, the DMMP staff and project proponents have discussed several possible program clarifications. These include establishing:

1. Guidelines for standard reporting of ammonia data
2. Threshold ammonia concentrations and guidelines for conducting ammonia reference toxicant ($LC_{50}$) tests
3. Threshold ammonia concentrations above which DMMP agencies will consider allowing purging of samples

4. Methods for purging ammonia from the overlying sample water and guidelines for test initiation after purging (batching)

## PROPOSED CLARIFICATIONS/ACTIONS

*1. Standard reporting of ammonia data*

The DMMP agencies propose that the following information be collected and reported for all test sediment where there is concern that ammonia toxicity may interfere with interpretation of test results, as well as appropriate control and reference samples, whether or not sample purging is allowed and then ultimately occurs.

- Total interstitial ammonia from the original bulk sediment sample

- Total and unionized interstitial ammonia at the start and end of each toxicity test, e.g., at day 0 and day 10

- All water-only ammonia reference toxicant test data ($LC_{50}$, total and unionized)

*2. Threshold ammonia concentrations and guidelines for conducting ammonia reference toxicant ($LC_{50}$) tests*

The DMMP agencies recommend the total interstitial ammonia concentrations presented in Table 1 below be used as thresholds above which project proponents should conduct water-only ammonia reference toxicant ($LC_{50}$) tests (see 2001 clarification). These ammonia concentrations are equal to one-half the value provided in Table 2. With the standard ammonia data (proposed above), synoptic ammonia $LC_{50}$ data help the DMMP agencies and project proponents determine the potential extent of ammonia-related toxicity and reduce the need to purge sediment samples. Labs should already be experienced in running reference toxicant tests, however, project sampling and analysis plans (SAPs) should include a specific discussion including the lab's protocol for ammonia testing and calculating a $LC_{50}$, should the ammonia reference toxicant test be required.

**Table 1.** Thresholds for conducting ammonia reference toxicant ($LC_{50}$) tests.

| **Interstitial Ammonia** (mg/L @ pH 7.7) | ***Ampelisca abdita*** | ***Eohaustorius estuarius*** | ***Rhepoxinius abronius*** |
|---|---|---|---|
| **Total** | **<15** | **<30** | **<15** |
| **Unionized** | **<0.2** | **<0.4** | **<0.2** |

*3. Threshold ammonia concentrations for consideration of sample purging*

The US EPA (1994) presents species-specific no-effect concentrations for interstitial ammonia (Table 2). The DMMP proposes using the total interstitial ammonia values listed in Table 2 as thresholds for consideration of test sediment purging. [a]

Project proponents concerned that ammonia toxicity may interfere with interpretation of sediment bioassays must measure total interstitial ammonia concentrations in bulk sediment samples prior to test set up and initiation. If the bulk sediment interstitial ammonia concentrations approach or exceed those listed in Table 2, then the proponent must immediately coordinate with the Dredged Material Management Office (DMMO), Seattle District U.S. Army Corps of Engineers and develop an acceptable plan for monitoring interstitial ammonia. The final decision whether or not to allow toxicity test sample purging prior to test initiation will be made by the DMMP agencies using best professional judgment, and in collaboration with the applicant. If purging is allowed and occurs, the project proponent would be required to collect and report the following additional information for each test sample and the associated control and reference samples.

- Total and unionized interstitial ammonia (mg/L) on each day interstitial ammonia is measured during purging

- Total and unionized interstitial ammonia (mg/L) on any additional test days, if proposed or required in the ammonia monitoring plan

[a] While unionized ammonia values should also be calculated, errors associated with temperature and pH measurements may lead to uncertainties in the calculation. Consistent with local labs and programs nationally, the DMMP proposes to use the total values in both Table 1 and Table 2.

**Table 2.** Thresholds for considering sample purging.

| **Interstitial Ammonia (mg/L @ pH 7.7)** | ***Ampelisca abdita*** | ***Eohaustorius estuarius*** | ***Rhepoxinius abronius*** |
|---|---|---|---|
| **Total** | **<30** | **<60** | **<30** |
| **Unionized** | **<0.4** | **<0.8** | **<0.4** |

From US EPA (1994)

It should be noted that some guidance suggests the same total ammonia interstitial threshold concentration is appropriate for all three of these amphipod species (US EPA/US ACOE, 1993). However, the DMMP agencies believe that a higher total interstitial ammonia threshold concentration for *Eohaustorius estuarius* is appropriate and consistent with higher $LC_{50}$ values published in the scientific literature compared to *Ampelisca abdita* and *Rhepoxinius abronius*, i.e., *E. estuarius* is less sensitive to ammonia (Kohn et al., 1994).

*4. Purging methods and test initiation*

The DMMP agencies propose to use best professional judgment for those projects where purging may be indicated. Currently there are a variety of approaches used by regulatory agencies, project proponents and laboratories to purge samples, measure interstitial ammonia and initiate toxicity tests. In general, if purging is performed, overlying water is replaced 2x per day. Frequency of testing of the interstitial water in sacrificial containers may vary but generally occurs every 1-3 days, depending on the length of time purging is likely to occur. Once test sediment has reached the desired interstitial ammonia level, the test may be initiated, and each test sediment must have associated and similarly purged control and reference sediments.

The above describes the general approach. However, should purging be pursued for a project, there are many ways to vary the purging of samples and test initiation for individual samples or batches of samples. The DMMP strategy for any particular project will be to minimize purging to the extent practical and will be based on the bulk interstitial ammonia values that are provided up front. Below are some potential options for tailoring a project-specific purging regime.

- Set a number of days purging may occur overall
- Set a number of days any sample may receive purging that is not required due to ammonia levels
- Batch groups of samples for test initiation -- this may be based on initial ammonia levels or on actual time taken to reach the desired ammonia level for testing (e.g. for a group of 10

samples, batch and initiate the first 5 samples that reach the desired ammonia level, then wait and initiate the final 5 samples together – each group having associated purged control and reference sediments)

Laboratories with purging experience can generally estimate, based on initial bulk interstitial total ammonia values, the purging time required to reduce interstitial ammonia levels to threshold concentrations. Once coordination with the DMMP has occurred and a test strategy has been developed, the labs can a) plan for procurement and acclimation of test organisms, b) sequence various batches for purging, and c) attempt to start toxicity tests, including those for samples that are not purged, at approximately the same time.

Finally, other experimental techniques for reducing interstitial ammonia levels have been explored nationally to attempt to provide methods that reduce ammonia levels in shorter amounts of time and better maintain the character of the original samples (Ferretti et al., 2000). Deviations from the current standard method of purging are not being proposed at this time.

## REFERENCES

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